CLEAN VERSION OF AMENDED SPECIFICATION PARAGRAPHS

METALLOPROTEINASE INHIBITORS FOR WOUND HEALING
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At page 2, the amended paragraph beginning on line 24 and continuing on page 3:

The present invention is directed to peptide inhibitors of matrix metalloproteinases. These peptide inhibitors have amino acid sequences identical or related to the linking region spanning the two globular domains of matrix metalloproteinases. Several types of matrix metalloproteinases and their sequences are known, including matrix metalloproteinase-1, matrix metalloproteinase-2, matrix metalloproteinase-3, matrix metalloproteinase-4, matrix metalloproteinase-5, matrix metalloproteinase-6, matrix metalloproteinase-7, matrix metalloproteinase-8, and matrix metalloproteinase-9, matrix metalloproteinase-10, matrix metalloproteinase-11, matrix metalloproteinase-12, and matrix metalloproteinase-13. The invention contemplates inhibitors having amino acid sequences from the linking region of any of the matrix metalloproteinases. For example, peptide inhibitors of the invention can have amino acid sequences drawn from any region from about amino acid 70 to about amino acid 120 of the matrix metalloproteinase-2 sequence (SEQ ID NO:14), and analogous regions of all other matrix metalloproteinases.

At page 3, the amended paragraph beginning on line 9 and continuing on page 4 :

The invention provides peptides of any one of formulae (I), (II), (III):

Xaa_1-Xaa_2-Xaa_3-Xaa_4-Xaa_5-Xaa_6 Xaa_7-Xaa_8-Xaa_9 (I)

Xaa_{10}-Xaa_{11}-Xaa_{12} Xaa_{13}-Xaa_{14}-Xaa_{15}-Xaa_{16}-Xaa_{17}-Xaa_{18}-Xaa_{19} (II)

Xaa_1-Xaa_2-Xaa_3-Xaa_4-Xaa_5-Xaa_6-Xaa_7-Xaa_8-Xaa_9-Xaa_{10}-Xaa_{11}-
Xaa_{12}-Xaa_{13}-Xaa_{14}-Xaa_{15}-Xaa_{16}-Xaa_{17}-Xaa_{18}-Xaa_{19} (III)

wherein

Xaa_1, Xaa_4, and Xaa_6 are separately each apolar amino acids;
Xaa_2 is a basic amino acid;
Xaa_3 is a cysteine-like amino acid;
Xaa_5 is a polar or aliphatic amino acid;
Xaa_7 is an acidic amino acid,
Xaa_8 is an aliphatic or polar amino acid;
Xaa_9 is an aliphatic, apolar or basic amino acid; and

Xaa_{10} is a polar, acidic, basic or apolar amino acid;
Xaa_{11} is a polar or aromatic amino acid;
Xaa_{12} is a polar, basic, aliphatic or apolar amino acid ;
Xaa_{13} is an aromatic, aliphatic, polar or acidic amino acid;
Xaa_{14} is an aromatic, apolar or polar amino acid;
Xaa_{15} is an apolar or acidic amino acid;
Xaa_{16} is a basic, a polar or an apolar amino acid;
Xaa_{17} is a basic, a polar, an aliphatic, an apolar or an acidic amino acid;
Xaa_{18} is an apolar or an aliphatic amino acid;
Xaa_{19} is a basic or an aliphatic amino acid; and
wherein the peptide is capable of inhibiting the activity of matrix metalloproteinase-1, matrix metalloproteinase-2, matrix metalloproteinase-3, matrix metalloproteinase-4, matrix metalloproteinase-5, matrix metalloproteinase-6, matrix metalloproteinase-7, matrix metalloproteinase-8, or matrix metalloproteinase-9, matrix metalloproteinase-10, matrix metalloproteinase-11, matrix metalloproteinase-12, and matrix metalloproteinase-13. In a preferred embodiment, the peptide can inhibit the activity of matrix metalloproteinase-2, matrix metalloproteinase-3, matrix metalloproteinase-7, matrix metalloproteinase-8, or matrix metalloproteinase-9.

At page 5, the amended paragraph at line 1 and continuing on page 6:

The invention also provides peptides of formula (IV) (SEQ ID NO:18):

$$Xaa_a\text{-}Xaa_b\text{-}Xaa_c\text{-}Xaa_d\text{-}Xaa_e\text{-}Xaa_f\text{-}Xaa_g\text{-}Xaa_h\text{-}Xaa_i\text{-}Xaa_j\text{-}Xaa_k\text{-}Xaa_L\text{-}Xaa_m\text{-}Xaa_1\text{-}Xaa_2\text{-}Xaa_3\text{-}Xaa_4\text{-}Xaa_5\text{-}Xaa_6\text{-}Xaa_7\text{-}Xaa_8\text{-}Xaa_9\text{-}Xaa_{10}\text{-}Xaa_{11}\text{-}Xaa_{12}\text{-}Xaa_{13}\text{-}Xaa_{14}\text{-}Xaa_{15}\text{-}Xaa_{16}\text{-}Xaa_{17}\text{-}Xaa_{18}\text{-}Xaa_{19} \quad \text{(IV)}$$

wherein:

Xaa_a is proline;
Xaa_b is glutamine or glutamic acid;
Xaa_c is threonine;
Xaa_d is glycine;
Xaa_e is aspartic acid or glutamic acid;
Xaa_f is leucine;
Xaa_g is aspartic acid;
Xaa_h is glutamine or serine;
Xaa_i is asparagine or alanine;
Xaa_j is threonine;
Xaa_k is isoleucine or leucine;

Xaa_1 is proline;
Xaa_2 is arginine;
Xaa_3 is cysteine;
Xaa_4 is glycine;
Xaa_5 is valine or asparagine;
Xaa_6 is proline;
Xaa_7 is aspartic acid;
Xaa_8 is valine or leucine;
Xaa_9 is alanine or glycine;
Xaa_{10} is asparagine or arginine;
Xaa_{11} is tyrosine or phenylalanine;

Xaa_L is glutamic acid or lysine;
Xaa_m is threonine or alanine;
Xaa_n is methionine;
Xaa_o is arginine;
Xaa_p is lysine or threonine;
Xaa_{12} is asparagine or glutamine;
Xaa_{13} is phenylalanine or threonine;
Xaa_{14} is phenylalanine;
Xaa_{15} is proline or glutamic acid;
Xaa_{16} is arginine or glycine;
Xaa_{17} is lysine or aspartic acid;
Xaa_{18} is proline or leucine;
Xaa_{19} is lysine; and

wherein the peptide is capable of inhibiting the activity of a metalloproteinase. For example, the metalloproteinase can be matrix metalloproteinase-1, matrix metalloproteinase-2, matrix metalloproteinase-3, matrix metalloproteinase-4, matrix metalloproteinase-5, matrix metalloproteinase-6, matrix metalloproteinase-7, matrix metalloproteinase-8, and matrix metalloproteinase-9, matrix metalloproteinase-10, matrix metalloproteinase-11, matrix metalloproteinase-12, or matrix metalloproteinase-13. Desirable peptides inhibit matrix metalloproteinase-2 or matrix metalloproteinase-9.

At page 6, the amended paragraph beginning on line 29 and continuing on pages 7 and 8:

The invention further provides a method for treating a wound that comprises administering a therapeutically effective amount of a peptide of formula I, II, III or IV :

$$Xaa_1\text{-}Xaa_2\text{-}Xaa_3\text{-}Xaa_4\text{-}Xaa_5\text{-}Xaa_6\ Xaa_7\text{-}Xaa_8\text{-}Xaa_9 \quad \text{(I)}$$

$$Xaa_{10}\text{-}Xaa_{11}\text{-}Xaa_{12}\ Xaa_{13}\text{-}Xaa_{14}\text{-}Xaa_{15}\text{-}Xaa_{16}\text{-}Xaa_{17}\text{-}Xaa_{18}\text{-}Xaa_{19} \quad \text{(II)}$$

$$Xaa_1\text{-}Xaa_2\text{-}Xaa_3\text{-}Xaa_4\text{-}Xaa_5\text{-}Xaa_6\text{-}Xaa_7\text{-}Xaa_8\text{-}Xaa_9\text{-}Xaa_{10}\text{-}Xaa_{11}\text{-}Xaa_{12}\text{-}Xaa_{13}\text{-}Xaa_{14}\text{-}Xaa_{15}\text{-}Xaa_{16}\text{-}Xaa_{17}\text{-}Xaa_{18}\text{-}Xaa_{19} \quad \text{(III)}$$

$$Xaa_a\text{-}Xaa_b\text{-}Xaa_c\text{-}Xaa_d\text{-}Xaa_e\text{-}Xaa_f\text{-}Xaa_g\text{-}Xaa_h\text{-}Xaa_i\text{-}Xaa_j\text{-}Xaa_k\text{-}Xaa_L\text{-}Xaa_m\text{-}Xaa_1\text{-}Xaa_2\text{-}Xaa_3\text{-}Xaa_4\text{-}Xaa_5\text{-}Xaa_6\text{-}Xaa_7\text{-}Xaa_8\text{-}Xaa_9\text{-}Xaa_{10}\text{-}Xaa_{11}\text{-}Xaa_{12}\text{-}Xaa_{13}\text{-}Xaa_{14}\text{-}Xaa_{15}\text{-}Xaa_{16}\text{-}Xaa_{17}\text{-}Xaa_{18}\text{-}Xaa_{19} \quad \text{(IV)}$$

(SEQ ID NO:20)

wherein:

Xaa_1, Xaa_4, and Xaa_6 are separately each apolar amino acids;
Xaa_2 is a basic amino acid;
Xaa_3 is a cysteine-like amino acid;
Xaa_5 is a polar or aliphatic amino acid;
Xaa_7 is an acidic amino acid,
Xaa_8 is an aliphatic or polar amino acid;
Xaa_9 is an aliphatic, apolar or basic amino acid; and

Xaa_{10} is a polar, acidic, basic or apolar amino acid;
Xaa_{11} is a polar or aromatic amino acid;
Xaa_{12} is a polar, basic, aliphatic or apolar amino acid ;
Xaa_{13} is an aromatic, aliphatic, polar or acidic amino acid;
Xaa_{14} is an aromatic, apolar or polar amino acid;
Xaa_{15} is an apolar or acidic amino acid;
Xaa_{16} is a basic, a polar or an apolar amino acid;
Xaa_{17} is a basic, a polar, an aliphatic, an apolar or an acidic amino acid;
Xaa_{18} is an apolar or an aliphatic amino acid;
Xaa_{19} is a basic or an aliphatic amino acid;
Xaa_a is proline;
Xaa_b is glutamine or glutamic acid;
Xaa_c is threonine;
Xaa_d is glycine;
Xaa_e is aspartic acid or glutamic acid;
Xaa_f is leucine;
Xaa_g is aspartic acid;
Xaa_h is glutamine or serine;
Xaa_i is asparagine or alanine;
Xaa_j is threonine;
Xaa_k is isoleucine or leucine;
Xaa_L is glutamic acid or lysine;
Xaa_m is threonine or alanine;
Xaa_n is methionine;
Xaa_o is arginine; and
Xaa_p is lysine or threonine;

wherein the peptide is capable of inhibiting the activity of a matrix metalloproteinase.

At page 9, the amended paragraph 2:

Figure 1 provides a CLUSTAL X (version 1.8) multiple sequence (SEQ ID NOS:2-10) alignment of the cleavage spanning regions of select MMP proenzymes. Figure 1A provides an alignment that highlights conserved residues where an '*' indicates complete identity among the sequences, a ':' indicates 7/9 conserved positions, and a '.' indicates greater than 80% identical positions with mostly conserved substitutions. Figure 1B indicates the positions of heterogeneity in bold.

At page 25, the paragraph beginning on line 9 and continuing on page 26:

In a preferred embodiment (SEQ ID NO:19):

Xaa_1 is proline,
Xaa_2 is arginine,
Xaa_3 is cysteine,
Xaa_4 is glycine,
Xaa_5 is valine or asparagine,
Xaa_6 is proline,
Xaa_7 is aspartic acid,
Xaa_8 is valine, leucine, or serine,
Xaa_9 is alanine, glycine or histidine,
Xaa_{10} is asparagine, aspartic acid, histidine, arginine, glutamine or glycine,
Xaa_{11} is tyrosine or phenylalanine,
Xaa_{12} is asparagine, serine, arginine, glutamine, valine or methionine,
Xaa_{13} is phenylalanine, valine, leucine, threonine, serine, or glutamic acid,
Xaa_{14} is phenylalanine, methionine or threonine,
Xaa_{15} is proline or glutamic acid,
Xaa_{16} is arginine, asparagine or glycine,
Xaa_{17} is lysine, threonine, serine, isoleucine, methionine, glycine, aspartic acid or asparagine,
Xaa_{18} is proline or leucine, and
Xaa_{19} is lysine, valine or arginine.

At page 27, the paragraph beginning on line 13 and continuing on page 28:

In one embodiment, it is desirable to inhibit MMPs-2 and 9, but to keep the level of MMP-1 relatively unregulated in order to heal chronic wounds. Based on the sequence alignment above one of skill in the art can design a peptide with amino acids that are found in MMP2 and MMP9 proenzyme sequences but not in the MMP1 proenzyme sequence, to produce a peptide that will inhibit MMPs-2 and 9, while leaving MMP-1 uninhibited. Such a peptide is provided by formula IV.

$$Xaa_a\text{-}Xaa_b\text{-}Xaa_c\text{-}Xaa_d\text{-}Xaa_e\text{-}Xaa_f\text{-}Xaa_g\text{-}Xaa_h\text{-}Xaa_i\text{-}Xaa_j\text{-}Xaa_k\text{-}Xaa_L\text{-}Xaa_m\text{-}Xaa_1\text{-}Xaa_2\text{-}Xaa_3\text{-}Xaa_4\text{-}Xaa_5\text{-}Xaa_6\text{-}Xaa_7\text{-}Xaa_8\text{-}Xaa_9\text{-}Xaa_{10}\text{-}Xaa_{11}\text{-}Xaa_{12}\text{-}Xaa_{13}\text{-}Xaa_{14}\text{-}Xaa_{15}\text{-}Xaa_{16}\text{-}Xaa_{17}\text{-}Xaa_{18}\text{-}Xaa_{19} \quad \text{(IV)}$$

(SEQ ID NO:18)

wherein:

Xaa_a is proline;
Xaa_b is glutamine or glutamic acid;
Xaa_c is threonine;
Xaa_1 is proline;
Xaa_2 is arginine;
Xaa_3 is cysteine;

Xaa_d is glycine;
Xaa_e is aspartic acid or glutamic acid;

Xaa_f is leucine;
Xaa_g is aspartic acid;
Xaa_h is glutamine or serine;

Xaa_i is asparagine or alanine;

Xaa_j is threonine;
Xaa_k is isoleucine or leucine, preferably isoleucine;
Xaa_L is glutamic acid or lysine, preferably glutamic acid;
Xaa_m is threonine or alanine;
Xaa_n is methionine;

Xaa_o is arginine;

Xaa_p is lysine or threonine;
Xaa_{17} is lysine or aspartic acid;
Xaa_{19} is lysine.

Xaa_4 is glycine;
Xaa_5 is valine or asparagine, preferably asparagine;
Xaa_6 is proline;
Xaa_7 is aspartic acid;
Xaa_8 is valine or leucine, preferably leucine;
Xaa_9 is alanine or glycine, preferably glycine;
Xaa_{10} is asparagine or arginine;
Xaa_{11} is tyrosine or phenylalanine, preferably tyrosine;
Xaa_{12} is asparagine or glutamine;
Xaa_{13} is phenylalanine or threonine;
Xaa_{14} is phenylalanine;
Xaa_{15} is proline or glutamic acid, preferably proline;
Xaa_{16} is arginine or glycine, preferably arginine;
Xaa_{18} is proline or leucine, preferably leucine; and